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(54) Title: METHODS FOR SPATIALLY-DISPERSED POSITIONALLY-ENCODED COMBINATORIAL LIBRARY SYNTHESIS

## (57) Abstract

The present invention relates to a method useful in combinatorial chemistry. More specifically, the present invention relates to methods for synthesizing spatially-dispersed positionally-encoded combinatorial chemistry libraries of oligomers whereby the synthesis is carried out on a plurality of solid supports which in turn are distributed in the form of a series of arrays. The position of each solid support in each array determines the exact identity of the oligomer.

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Methods for Spatially-Dispersed Positionally-Encoded  
Combinatorial Library Synthesis

Field of the Invention

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The present invention relates to a method useful in combinatorial chemistry. More particularly, the present invention relates to methods for synthesizing spatially-dispersed positionally-encoded combinatorial chemistry libraries of oligomers whereby the synthesis is carried out on a plurality of solid supports which in turn are distributed in the form of a series of arrays. The position of each solid support in each array determines the exact identity of the oligomer.

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Background of the Invention

The screening of chemical libraries to identify compounds which have novel pharmacological<sup>1</sup> and material science properties<sup>2</sup> is a common practice. These chemical libraries may be a collection of structurally related oligopeptides, oligonucleotides, small or large molecular weight organic or inorganic molecules. Those practiced in the art of combinatorial chemistry can accomplish the synthesis of combinatorial chemical libraries using two general methods. These methods are known to those skilled in the art as "spatially-addressable" methods and "split-pool" methods. It is common to practice these methods using solid support chemical synthesis techniques as discussed by Gordon, et. al.<sup>3</sup>

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A common feature to the spatially-addressable combinatorial library methods is that a unique combination of monomers is reacted to form a single oligomer or compound or, alternately, set of oligomers or compounds at a predefined unique physical location or address in the synthesis process. An example of the spatially-addressable method is provided by Geysen et. al.<sup>4</sup> and involves the generation of peptide libraries on an array of immobilized polymeric pins (a solid support) that fit the dimensions of a 96-well microtiter plate. A two-dimensional matrix of combinations is generated in each microtiter plate

experiment, where  $n \times m$  unique oligomers or compounds are produced for a combination of  $n + m$  parallel monomer addition steps. The structure of each of the individual library members is determined by analyzing the pin location and the monomers employed at that address during the sequence of reaction steps in the synthesis. An advantage of this method is that individual oligomer or compound products can be released from the polymeric pin surface in a spatially-addressable manner to allow isolation and screening of each discrete member of the library. Another advantage of this method is that the number of solid supports required is equal to, i.e. *no larger than*, the number of library members to be synthesized. Thus, relatively large quantities, i.e. micromolar quantities, of individual library members are synthesized in a practical manner using this method. Related to the Geysen pin method are the parallel synthesis methods which use a reaction vessel system such as that practiced by Cody, et al.<sup>5</sup> This is the practice of distributing a quantity of solid support, such as chemically-derivatized polymeric resin beads (namely those of the composition polystyrene, polystyrene grafted with polyethylene glycol, or polyacrylimide, etc.) in a two dimensional matrix of  $n \times m$  individual reaction vessels allowing the parallel addition of a set of  $n \times m$  reactive monomers to produce a set of  $n \times m$  oligomers or compounds. This spatially-addressable method has advantages similar to that of Geysen, et. al. Thus, individual oligomer or compound products can be released from the solid support in a spatially-addressable manner to allow isolation and screening of each discrete member of the library. Additionally, the number of solid supports required is equal to, i.e. *no larger than*, the number of library members to be synthesized. Thus, relatively large quantities, i.e. micromolar to millimolar quantities, of individual library members also are synthesized in a practical manner using this method. Another example of a spatially-addressable method is the photolithographic method for synthesizing a collection of oligomers or compounds on the chemically-derivatized surface of a chip (a solid support) provided by Fodor et. al.<sup>6</sup> A variety of masking strategies can be employed to selectively remove photochemically-labile protecting groups thus revealing reactive functional groups at defined spatial locations on the chip. The

functional groups are reacted with a monomer by exposing the chip surface to appropriate reagents. The sequential masking and reaction steps are recorded, thus producing a pre-defined record of discrete oligomers or compounds at known spatial addresses in an experiment. An advantage of this method is that binary masking strategies can be employed to produce  $2^n$  unique oligomers or compounds for  $n$  masking and monomer addition cycles. Two important disadvantages of this method are that a) relatively minute quantities are produced on the surface of the chip and; b) release and isolation of individual library members is not technically feasible.

Split-pool combinatorial library methods differ from spatially-addressable methods in that the physical location of each unique oligomer or compound is not discrete. Instead, pools of library members are manipulated throughout the experiment. There are two major categories of split-pool methods currently in practice. These are: 1) deconvolution methods<sup>7</sup> pioneered by Furka et. al.<sup>8</sup> and Houghten, et. al.<sup>9</sup> and 2) encoded methods<sup>10</sup> by Gallop et. al., Still, et. al. and others.

It is common in the practice to employ solid support-based chemistry for these methods. A collection of solid supports are split into individual pools. These pools are then exposed to a series of reactive monomers, followed by a recombination step, in which the position of all solid supports is randomized. The solid supports are then split into a new set of individual pools, exposed to a new series of reactive monomers, followed by a second recombination step. By repeating this split, react and recombine process all possible combinations of oligomers or compounds from the series of monomers employed are obtained, *providing a large excess of solid supports are utilized*.<sup>11</sup> The number of oligomers or compounds obtained in an experiment is equal to the *product of the monomers employed*, however, the number of chemical transformation steps required is only equal to the *sum of the monomers employed*. Therefore, a geometric amplification of oligomers or compounds is realized relative to the amount of chemical transformation steps employed. For instance, only nine (9) transformation steps were employed using three (3) amino acid

monomers in a three step process for the combinatorial synthesis of 27 peptide oligomers.<sup>12</sup>

The prior art split-pool methods produce pools of oligomers or compounds as a product of the experiment. Therefore, the  
5 identification of a specific member of the library is typically found by screening the pools for a desired activity, biological or otherwise. The disadvantages of the deconvolution split-pool methods are that (a) the technique always requires that large mixtures of oligomers are screened in bioassays, (b) sequential  
10 rounds of resynthesis and bioassay are always required to deconvolute a library, and (c) since single oligomers are not produced a library is always stored as a mixture, requiring later deconvolution. In the practice of encoded split-pool methods physical separation of the solid support is required to accomplish  
15 two tasks: first, to physically isolate the individual library member after screening and, second, to de-code the identity of the tag and thus deduce the chemical structure of the member. A disadvantage specific to the chemically encoded split-pool methods is that chemical tags introduce potential side reactions and  
20 failures both with orthogonal linkers and with tags, thus requiring compatibility between the tag chemistry and the chemistry utilized to synthesize the combinatorial library.

In practice, both categories of split-pool methods require a large excess of solid support beads to ensure with reasonable  
25 certainty (99% confidence level) that all possible oligomers are made when a random split-pool strategy is employed.<sup>13</sup> This is necessary because the exact identity of each bead (i.e. the identity of each oligomer) is lost due to the unstructured nature of the split-pool method. This presents a significant problem when  
30 scaling up these methods for the production of micromole or larger amounts of individual oligomers in the library.

There is a need in the combinatorial chemistry art for a technique which can achieve geometric amplification in the  
35 number of library members synthesized relative to the number of synthetic steps required but, additionally, (a) avoid chemical encoding steps (b) produce micromolar or larger amounts of individual oligomers; (c) use only the number of solid supports

required for the number of possible oligomers in the library; and  
(d) produce the oligomers in spatially-dispersed arrays.

5           Glossary

Monomer: As used herein, a "monomer" is any atom or  
          molecule capable of forming at least one chemical bond. Thus, a  
          "monomer" is any member of the set of atoms or molecules that  
          can be joined together as single units in a multiple of sequential or  
10           concerted chemical or enzymatic reaction steps to form an  
          oligomer. Monomers may have one or a plurality of functional  
          groups, which functional groups may be, but need not be,  
          identical. The set of monomers useful in the present invention  
          includes, but is not restricted to, alkyl and aryl amines; alkyl and  
15           aryl mercaptans; alkyl and aryl ketones; alkyl and aryl carboxylic  
          acids; alkyl and aryl esters; alkyl and aryl ethers; alkyl and aryl  
          sulfoxides; alkyl and aryl sulfones; alkyl and aryl sulfonamides;  
          phenols; alkyl alcohols; alkyl and aryl alkenes; alkyl and aryl  
          lactams; alkyl and aryl lactones; alkyl and aryl di- and polyenes;  
20           alkyl and aryl alkynes; alkyl and aryl unsaturated ketones; alkyl  
          and aryl aldehydes; heteroatomic compounds containing one or  
          more of the atoms of: nitrogen, sulfur, phosphorous, oxygen, and  
          other polyfunctional molecules containing one or more of the above  
          functional groups; L-amino acids; D-amino acids;  
25           deoxyribonucleosides; deoxyribonucleotides; ribonucleosides;  
          ribonucleotides; sugars; benzodiazepines;  $\beta$ -lactams; hydantoins;  
          quinones; hydroquinones; terpenes; and the like. The monomers of  
          the present invention may have groups protecting the functional  
          groups within the monomer. Suitable protecting groups will  
30           depend on the functionality and particular chemistry used to  
          construct the library. Examples of suitable functional protecting  
          groups will be readily apparent to skilled artisans, and are  
          described, for example, in Greene and Wuts,<sup>14</sup> which is  
          incorporated herein by reference. As used herein, "monomer"  
35           refers to any member of a basis set for synthesis of an oligomer.  
          For example, the dimers of 20 L-amino acids form a basis set of  
          400 "monomers" for synthesis of polypeptides. Different basis sets

of monomers may be used at successive steps in the synthesis of an oligomer.

Oligomer: As used herein, an "oligomer" is any chemical structure that can be synthesized using the combinatorial library methods of this invention, including, for example, amides, esters, thioethers, ketones, ethers, sulfoxides, sulfonamides, sulfones, phosphates, alcohols, aldehydes, alkenes, alkynes, aromatics, polyaromatics, heterocyclic compounds containing one or more of the atoms of: nitrogen, sulfur, oxygen, and phosphorous, and the like; chemical entities having a common core structure such as, for example, terpenes, steroids,  $\beta$ -lactams, benzodiazepines, xanthates, indoles, indolones, lactones, lactams, hydantoins, quinones, hydroquinones, and the like; chains of repeating monomer units such as polysaccharides, phospholipids, polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polyimides, polyacetates, polypeptides, polynucleotides, and the like; or other oligomers as will be readily apparent to one skilled in the art upon review of this disclosure. Thus, an "oligomer" of the present invention may be linear, branched, cyclic, or assume various other forms as will be apparent to those skilled in the art. Thus, "oligomer" may be used synonymously or interchangeably with "compound", thus describing any structure, organic or inorganic, which can be produced in a sequential fashion via the addition of monomeric units as described above.

Solid Support: A "solid support" is a material, or combination of materials, having a rigid or semi-rigid surface and having functional groups or linkers, or that is capable of being chemically derivatized with functional groups or linkers, that are suitable for carrying out chemical synthesis reactions. Such materials will preferably take the form of small beads, pellets, disks, capillaries, hollow fibers, needles, solid fibers, cellulose beads, pore-glass beads, silica gels, polystyrene beads cross-linked with divinylbenzene and optionally grafted with polyethylene glycol, grafted co-poly beads, polyacrylamide beads, latex beads, dimethylacrylamide beads optionally cross-linked with N,N'-bis-acryloyl ethylene diamine, polydimethylacrylamide beads crosslinked with polystyrene, glass particles coated with a



hydrophobic polymer, or other convenient forms. "Solid supports" may be constructed such that they are capable of being transferred mechanically from one support carrier to another support carrier.

5        Linker: A "linker" is a moiety, molecule, or group of molecules attached to a solid support and spacing a synthesized oligomer from the solid support. Typically a linker will be bi-functional, wherein said linker has a functional group at one end capable of attaching to a monomer, oligomer, or solid support, a series of spacer residues, and a functional group at another end capable of  
10        attaching to a monomer, oligomer, or solid support. The functional groups may be, but need not be, identical. Additionally, said linker may be cleaved by a chemical transformation such that the synthesized oligomer, or part of the synthesized oligomer, or the synthesized oligomer and the linker, or the synthesized oligomer  
15        and part of the linker may be chemically separated from the solid support, linker, or both.

#### Summary of the Invention

20        An object of the present invention is to provide a method for the synthesis of a spatially-dispersed combinatorial library of oligomers, in which the oligomers are distributed in a controlled manner. These oligomers are comprised of a series of monomers  
25        which are introduced into the oligomers in a sequential and stepwise fashion via chemical transformation steps (hereafter referred to as "steps"). These monomers are comprised of subsets of monomers such that the first subset of monomers is introduced in the first step, the second set of monomers is introduced in the  
30        second step, etc. The method further describes a means for introducing the monomers in a sequential and stepwise fashion on a series of solid supports. The number of supports equals the number of oligomers in the library.

35        A novel aspect of this process as distinguished from the prior art is that the supports are arranged in, and subsequently redistributed in a controlled manner between, a series of arrays. This series of arrays provide a means for holding the supports in physically discrete locations such that the exact identity of each

support is provided by its location. The series of arrays of supports are placed in a further series of reaction vessels for the individual steps of an oligomer synthesis. After each step in the oligomer synthesis the supports are redistributed from one series of arrays to a next series of arrays.

A further novel aspect of this process is that between each step the redistribution of the supports is carried out in an controlled fashion, such that all possible combinations of possible oligomers are synthesized. A further novel aspect of this process is that the positions of all supports are known during the synthesis experiment such that the identity of an oligomer is unequivocally established by its physical location. Thus, the applied method achieves a geometric amplification in the number of library members synthesized relative to the number of synthetic steps required while providing individual library members in a spatially-dispersed format. Thus, the use of a tagging system is eliminated for a split-pool synthesis experiment.

The method has utility in the production of oligomers which are available for screening in assays for novel biological, chemical, or physical properties which may have commercial value. Further, the structures of these oligomers are readily identifiable by virtue of their physical location. The method further provides a means for producing each oligomer in a discrete physical location which allows any pre-determined oligomer to be readily isolated from all other oligomers in the library. Yet another advantage of the invention is that no excess of solid supports is required, thus enabling a larger scale of synthesis.

#### Detailed Description of the Invention

##### A Library Synthesis Using Symmetrical Arrays

The method described herein is suitable for the synthesis of a library of oligomers comprised of two, three, or more sets of oligomers. However, the method described is most suitable for the synthesis of a library of oligomers comprised of three monomer subsets. The sums of monomers in each of these subsets may be variable. However, conveyance of the methodology to those practiced in the art may be initially illustrated when the sum of all

monomers in each subset is equal. The sum of monomers in each subset is defined by  $n$  in which  $n$  is a positive integer. The solid supports are preferably arranged in a series of arrays of dimension  $n \times n$ . There are a total of  $n$  such arrays required for the synthesis of such a library. Therefore, the total number of oligomers in a three step synthesis is the product  $n \times n \times n$ . The  $n$  arrays of solid supports are preferably arranged in support carriers (hereafter referred to as "carriers"). Each carrier holds an  $n \times n$  array of solid supports. Alternatively, several carriers may be used to hold an  $n \times n$  array of supports. However, preferably the carriers are of sufficient size to hold an entire  $n \times n$  array of supports. Thus, preferably there are  $n$  carriers which are placed in  $n$  reaction vessels for each chemical transformation step such that each individual monomer from a subset of  $n$  monomers is reacted with the supports in each individual reaction vessel.

A further novel aspect of this process as distinguished from the prior art is the method by which the supports are redistributed from one series of arrays to the next series of arrays between chemical transformation steps. This novel method is illustrated in Figure 1 for a three-step combinatorial synthesis using three subsets of monomers, each subset containing three monomers (i.e.  $n=3$ ), to produce a library of 27 (i.e.  $n \times n \times n=27$ ) oligomers. The supports are arranged as arrays on the carriers and reacted with a first subset of monomers. After chemical transformation step #1, the columns of supports in the first series of arrays are redistributed such that the first column from the first array in the first series is transferred to the first column of the first array in the second series; the second column from first array in the first series is transferred to the first column of the second array in the second series; the third column from the first array in the first series is transferred to the first column of the third array in the second series. The first, second and third columns of supports from the second and third arrays in the first series are redistributed to the second series of arrays for chemical transformation step #2 in a similar fashion. Having completed this redistribution process, the arrays of supports undergo chemical transformation step #2. After step #2 the arrays are redistributed again to a new series of arrays.

Th method of redistribution is similar to that used after the chemical transformation step #1, however, it is the individual *rows* of each array that are redistributed rather than the *columns* described above. The redistribution is shown in Figure 1. Thus, the rows of supports in the second series of arrays are redistributed such that the first row from the first array in the second series is transferred to the first row of the first array in the third series; the second row from first array in the second series is transferred to the first row of the second array in the third series; the third row from the first array in the second series is transferred to the first row of the third array in the third series. The first, second and third rows of supports from the second and third arrays in the second series are redistributed to the third series of arrays for chemical transformation step #3 in a similar fashion. It should be noted that the redistribution of supports between the chemical transformation steps #1 and #2 and between steps #2 and #3 is functionally identical if one simply reorients the arrays such that rows become columns and columns become rows (such as accomplished by a 90 degree rotation). If such a reorientation of the second series of arrays occurs then the *columns* from the second series of arrays are redistributed to *columns* in the third series of arrays. Following this second redistribution, chemical transformation step #3 is carried out on the supports. Using this redistribution method, all 27 possible combinations of monomers are ensured thus producing a combinatorial library of 27 oligomers. It may be appropriate to indelibly mark the carriers which hold the arrays using a means to ensure that rows are recognized as distinct from columns in each array. Additionally, each carrier may also be indelibly marked to distinguish the contents of its arrays uniquely from other carrier's arrays. Preferably, a barcode reading device may be used to query this information from a barcode placed across the top of the columns or beside the rows of each carrier. As appreciated by those familiar in the art, this redistribution method for an  $n \times n \times n$  library is efficient, transferring entire columns or entire rows of supports simultaneously. The synthesis of these 27 oligomers was accomplished with three chemical transformation steps involving a total of only 3 individual reaction vessels for each step. The

products from the library synthesis experiment are held in discrete locations, thus allowing for the identification and isolation of each individual library member. Additionally, this redistribution method is amenable to automation via robotics.

5       The above illustration describes a method for synthesizing a library of oligomers of very modest size. The technique is readily extrapolated to the synthesis of much larger libraries, e.g. one million oligomers using a three step synthesis with arrays of dimension 100 x 100 supports, 100 carriers, 100 monomers in  
10       each step. Thus a total of 100 reaction vessels can be employed to produce a library of one million members, all spatially-dispersed, individually identifiable and individually isolated. Those practiced in the art will appreciate that spatially-dispersed techniques as previously practiced by Geysen, Cody, and Ellman<sup>15</sup> would have  
15       required 3 million reaction vessels to produce an identical resulting oligomer library.

#### A Library Synthesis Using Unsymmetrical Arrays. Case 1.

20       The method described herein is also most suitable for the synthesis of a library of oligomers comprised of three monomer subsets. The sums of monomers in each of these subsets may be variable. The sum of monomers in each subset is defined by  $m$ ,  $n$ , and  $p$ , respectively for the three subsets such that  $n$ ,  $m$ , and  $p$  are positive integers. The total number of unique oligomers produced  
25       in a three step synthesis is the product  $m \times n \times p$ . The solid supports are preferably arranged in a series of arrays of dimension  $n \times p$ . Therefore, a total of  $m$  such arrays are required for the first step in the synthesis of such a library. The  $m$  arrays of solid supports are preferably arranged in support carriers. Each carrier  
30       holds an  $n \times p$  array of solid supports. Alternatively, several carriers may be used to hold an  $n \times p$  array of supports. However, preferably the carriers are of sufficient size to hold an entire  $n \times p$  array of supports. Thus, preferably there are  $m$  carriers which are placed in  $m$  reaction vessels for the first chemical transformation  
35       step such that each individual monomer from a subset of  $m$  monomers is reacted with the supports in each individual reaction vessel.

A further novel aspect of this process as distinguished from the prior art is the method by which the supports are redistributed from one series of arrays to the next series of arrays between chemical transformation steps. This novel method is illustrated in Figure 2 for a three-step combinatorial synthesis using three subsets of monomers. The monomer subsets contain the sum  $m=3$  monomers in the first subset,  $n=2$  monomers in the second subset, and  $p=4$  monomers in the third subset to produce a library of 24 (i.e.  $m \times n \times p=24$ ) oligomers. The supports are arranged as  $m=3$  arrays of the dimension  $(2 \times 4)$  on the carriers and reacted with a first subset of monomers. After chemical transformation step #1, the columns of supports in the first series of arrays are redistributed such that the first column from the first array in the first series is transferred to the first column of the first array in the second series; the second column from first array in the first series is transferred to the first column of the second array in the second series; the third column from the first array in the first series is transferred to the first column of the third array in the second series; the fourth column from the first array of the first series is transferred to the first column of the fourth array of the second series. The first, second, third and fourth columns of supports from the second and third arrays in the first series are redistributed to the second series of arrays for chemical transformation step #2 in a similar fashion. The resulting  $p=4$  arrays have the dimension  $(2 \times 3)$ . Having completed this redistribution process, the arrays of supports undergo chemical transformation step #2. After step #2 the arrays are redistributed again to a new series of arrays. The method of redistribution is similar to that used after the chemical transformation step #1, however, it is the individual rows of each array that are redistributed rather than the columns described above. The redistribution is shown in Figure 2. Thus, the rows of supports in the second series of arrays are redistributed such that the first row from the first array in the second series is transferred to the first row of the first array in the third series; the second row from first array in the second series is transferred to the first row of the second array in the third series. The first and second rows of supports from the second, third, and fourth arrays in the second

series are redistributed to the third series of arrays for chemical transformation step #3 in a similar fashion. The resulting  $n=2$  arrays have the dimension  $(4 \times 3)$ . Having completed this redistribution process, the arrays of supports undergo chemical transformation step #3. It is noted that the redistribution of supports between the chemical transformation steps #1 and #2 and between steps #2 and #3 is functionally identical if one simply reorients the arrays such that rows become columns and columns become rows (such as accomplished by a 90 degree rotation). If such a reorientation of the second series of arrays occurs then the *columns* from the second series of arrays are redistributed to *columns* in the third series of arrays. Using this redistribution method, all possible combinations of monomers are ensured thus producing a combinatorial library of oligomers. As appreciated by those familiar in the art, this redistribution method for an  $m \times n \times p$  library is efficient, transferring entire columns or entire rows of supports simultaneously. Additionally, this redistribution method is amenable to automation via robotics.

#### A Library Synthesis Using Unsymmetrical Arrays. Case 2.

The method described herein is also most suitable for the synthesis of a library of oligomers comprised of three monomer subsets. Likewise, the sums of monomers in each of these subsets may be variable. The sum of monomers in each subset is defined by  $m$ ,  $n$ , and  $p$ , respectively for the three subsets such that  $n$ ,  $m$ , and  $p$  are positive integers. The total number of unique oligomers produced in a three step synthesis is the product  $m \times n \times p$ . The solid supports are preferably arranged in a series of arrays of dimension  $n \times p$ . Therefore, a total of  $m$  such arrays are required for the first step in the synthesis of such a library. The  $m$  arrays of solid supports are preferably arranged in support carriers. Each carrier holds an  $n \times p$  array of solid supports. Alternatively, several carriers may be used to hold an  $n \times p$  array of supports. However, preferably the carriers are of sufficient size to hold an entire  $n \times p$  array of supports. Thus, preferably there are  $m$  carriers which are placed in  $m$  reaction vessels for the first

chemical transformation step such that each individual monomer from a subset of  $m$  monomers is reacted with the supports in each individual reaction vessel.

5 A further novel aspect of this process as distinguished from the prior art is the method by which the supports are redistributed from one series of arrays to the next series of arrays between chemical transformation steps. This novel method is illustrated in Figure 3 for a three-step combinatorial synthesis using three subsets of monomers. The monomer subsets contain the sum  $m=3$  monomers in the first subset,  $n=2$  monomers in the second subset, 10 and  $p=4$  monomers in the third subset to produce a library of 24 (i.e.  $m \times n \times p=24$ ) oligomers. The supports are arranged as  $m=3$  arrays of the dimension  $(2 \times 4)$  on the carriers and reacted with a first subset of monomers. After chemical transformation step #1, 15 the rows of supports in the first series of arrays are redistributed such that the first row from the first array in the first series is transferred to the first row of the first array in the second series; the second row from first array in the first series is transferred to the first row of the second array in the second series. The first and 20 second rows of supports from the second and third arrays in the first series are redistributed to the second series of arrays for chemical transformation step #2 in a similar fashion. The resulting  $n=2$  arrays have the dimension  $(3 \times 4)$ . Having completed this redistribution process, the arrays of supports undergo 25 chemical transformation step #2. After step #2 the arrays are redistributed again to a new series of arrays. The method of redistribution is similar to that used after the chemical transformation step #1, however, it is the individual *columns* of each array that are redistributed rather than the *rows* described 30 above. The redistribution is shown in Figure 3. Thus, the columns of supports in the second series of arrays are redistributed such that the first column from the first array in the second series is transferred to the first column of the first array in the third series; the second column from first array in the second series is transferred to the first column of the second array in the 35 third series; the third column from the first array in the second series is transferred to the first column of the third array in the third series; the fourth column from the first array of the second



series is transferred to the first column of the fourth array of the third series. The first, second, third and fourth columns of supports from the second array in the second series are redistributed to the third series of arrays for chemical transformation step #3 in a similar fashion. The resulting  $p=4$  arrays have the dimension  $(3 \times 2)$ . Having completed this redistribution process, the arrays of supports undergo chemical transformation step #3. It is noted that the redistribution of supports between the chemical transformation steps #1 and #2 and between steps #2 and #3 is functionally identical if one simply reorients the arrays such that rows become columns and columns become rows (such as accomplished by a 90 degree rotation). If such a reorientation of the second series of arrays occurs then the *columns* from the second series of arrays are redistributed to *columns* in the third series of arrays. Using this redistribution method, all possible combinations of monomers are ensured thus producing a combinatorial library of oligomers. As appreciated by those familiar in the art, this redistribution method for an  $m \times n \times p$  library is efficient, transferring entire columns or entire rows of supports simultaneously. Additionally, this redistribution method is amenable to automation via robotics.

The methods heretofore have illustrated a means by which a library of the quantity  $(m \times n \times p)$  oligomers is synthesized by organizing a quantity of solid supports equal to the quantity  $(m \times n \times p)$  in a series of arrays such that: Case 1.  $m$  arrays of dimension  $(n \times p)$  are reacted with  $m$  monomers, then redistributed to  $p$  arrays of dimension  $(n \times m)$  and reacted with  $p$  monomers, then redistributed to  $n$  arrays of dimension  $(p \times m)$  and reacted with  $n$  monomers to produce a library of the quantity  $(m \times n \times p)$  oligomers; and Case 2.  $m$  arrays of dimension  $(n \times p)$  are reacted with  $m$  monomers, then redistributed to  $n$  arrays of dimension  $(m \times p)$  and reacted with  $n$  monomers, then redistributed to  $p$  arrays of dimension  $(m \times n)$  and reacted with  $p$  monomers. The methods described heretofore provide for definitive ways by which rows or columns of organized arrays of solid supports can be manipulated to ensure the efficient chemical synthesis of all oligomers from a set of monomers. It is noted that the order of operations regarding

the transfer of one series of arrays of solid supports to the next series of arrays of solid supports via the parallel movement of the supports from rows to rows, rows to columns, columns to columns, or columns to rows, may be varied to accomplish the necessary redistribution of arrays.

A general code relating the physical location of an oligomer to its monomer sequence can be developed. For a set of  $M$  monomers  $\{1, 2, \dots, m\}$ ,  $N$  monomers  $\{1, 2, \dots, n\}$  and  $P$  monomers  $\{1, 2, \dots, p\}$  used in a three-step, spatially-dispersed positionally-encoded library to produce a series of  $n$  arrays of dimension  $(p \times m)$ , the sequence of any oligomer in any array can be defined by  $M_m - P_p - N_n$ , where  $m$  is both the column position number in the array and the monomer number in set  $M$ ,  $p$  is both the row position number and monomer number in the set  $P$ , and  $n$  is both the array position number and the monomer number in the set  $N$ .

Likewise, for a set of  $M$  monomers  $\{1, 2, \dots, m\}$ ,  $N$  monomers  $\{1, 2, \dots, n\}$  and  $P$  monomers  $\{1, 2, \dots, p\}$  used in a three-step, spatially-dispersed positionally-encoded library synthesis to produce a series of  $p$  arrays of dimension  $(m \times n)$ , the sequence of any oligomer in any array can be defined by  $M_m - N_n - P_p$ , where  $m$  is both the row position number in the array and the monomer number in set  $M$ ,  $n$  is both the column position number and monomer number in the set  $N$ , and  $p$  is both the array position number and the monomer number in the set  $P$ .

As will become clear to those skilled in the art, the techniques described here can be extended to synthesize combinatorial libraries in which more than three combinatorial steps are employed. In such cases it becomes difficult to visualize and describe the sequence of positional transformations of solid supports among the series of arrays. A computer algorithm can be designed which takes as input the goals of a synthetic experiment: namely, the desired number of combinatorial steps and the desired number of monomers used in each combinatorial step. The algorithm can then generate a map of the protocol required to satisfy the experimental goal. This map would contain the same information as that given in the figures used herein. In the event that the experimental goal can not be satisfied the algorithm would

suggest a protocol which would achieve a result as close as possible to the desired result.

The algorithm could be constructed to generate only those protocols that are consistent with a set of constraints imposed by an actual laboratory apparatus, for example, a fixed number of reaction vessels, carrier racks of a given dimension, and so forth. Such a computer algorithm would be useful for the practical application of the techniques disclosed herein. As a refinement of this method, such a computer algorithm could be designed to generate machine instructions for an automated synthetic apparatus which would perform the necessary chemical steps and positional transformations required to synthesize the desired combinatorial library.

In order to further illustrate the practice of the present invention, the following examples are included. While these examples illustrate methods for three combinatorial steps, the methods may be extended to synthesize combinatorial libraries which require more than three combinatorial steps as will become obvious to those persons skilled in the art.

#### **Examples:**

##### **I. A Library of 512 Peptides.**

The methods described above are very useful for the synthesis of a peptide library. The preparation of a library of 512 trimeric peptides may be prepared on a series of polystyrene-grafted polyethylene crowns (Chiron Mimotopes Pty. LTD., Victoria, Australia) which can serve as solid supports for the synthesis. These supports are available with a derivatized surface such that a Rink<sup>16</sup> handle presenting a reactive amine functionality is available for peptide couplings. A collection of 512 crowns with a 6.1 micromole/crown loading are chosen. The crowns are mounted on stems which, in turn, are mounted in an two-dimensional array-like format on crown holders (Chiron Mimotopes Pty. LTD). Eight individual crown holders (carriers) are individually labeled as S1.1, S1.2, S1.3, S1.4, S1.5, S1.6, S1.7, S1.8. Each crown holder

containing 64 crowns, arranged in an 8 x 8 array format, is prepared. Additionally, the orientation of each array in the crown holder is clearly marked such that the columns are defined as distinct from the rows. An indelible marking pen may be used for this purpose. Reaction vessels are used which are polypropylene or glass trays and are of a dimension sufficient to allow all crowns to be fully submerged in a reaction solvent. The following procedure is initiated:

Step 1. The crown holders are placed in a set of 8 individual reaction vessels. The reaction vessels are filled with a solution of 25 % piperidine in *N*-methylpyrrolidine (NMP). After 40 min the arrays are removed from the reaction vessels and the crowns are washed liberally with 100 mL of NMP and 200 mL of dichloromethane.

Step 2. Eight reaction vessels, labeled 1, 2, 3, ...through 8, are filled with a reaction mixture composed of the following: 200mM *N*-hydroxybenzotriazole (HOBt), 200mM 2-(1H-hydroxybenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 100 mM fluorenylmethyloxycarbonyl-amino acid, and 250 mM diisopropylethylamine (DIEA) in a solution of NMP. Eight amino acids, protected as fluorenylmethyl carbamates, are used. Each reaction vessel receives a unique amino acid from the list of: 1. glycine; 2. alanine; 3. phenylalanine; 4. leucine; 5. lysine; 6. glutamic acid; 7. serine; and 8. threonine. The crown holders are put into the appropriate vessels such that holder S1.1 is in vessel 1, holder S1.2 is in vessel 2, etc. After three hours the arrays are removed from the reaction vessels and the crowns are washed liberally with 100 mL of NMP and 200 mL of dichloromethane.

Step 3. Eight individual crown holders (carriers) are individually labeled as S2.1, S2.2, S2.3, S2.4, S2.5, S2.6, S2.7, S2.8. Additionally, the orientation of the crown holders is clearly marked such that the columns are defined as distinct from the rows. The 8 columns from each of the 8 arrays of the S1 series of crown holders are transferred to the appropriate columns in the 8 arrays of the S2 series of crown holders in a manner such that each of the 8 amino acids are now represented on a column of crowns in each of the arrays in the S2 series.

Step 4. Repeat Step 1. on the S2 series of crown holders.

Step 5. Repeat Step 2. on the S2 series of crown holders whereby the crown holders are put into the appropriate vessels such that holder S2.1 is in vessel 1, holder S2.2 is in vessel 2, etc.

5 Step 6. Eight individual crown holders (carriers) are individually labeled as S3.1, S3.2, S3.3, S3.4, S3.5, S3.6, S3.7, S3.8. Additionally, the orientation of the crown holders is clearly marked such that the columns are defined as distinct from the rows. The 8 rows from each of the 8 arrays of the S2 series of  
10 crown holders are transferred to the appropriate rows in the 8 arrays of the S3 series of crown holders in a manner such that 64 unique dipeptides are now represented on the crowns in each of the arrays in the S3 series.

Step 7. Repeat Step 1. on the S3 series of crown holders.

15 Step 8. Repeat Step 2. on the S3 series of crown holders whereby the crown holders are put into the appropriate vessels such that holder S3.1 is in vessel 1, holder S3.2 is in vessel 2, etc.

Step 9. Repeat Step 1. on the S3 series of crown holders.

Step 10. The crown holders are positioned such that the  
20 crowns fit into the wells of eight 96 2 mL-well polypropylene microtiter plates (available from Beckman Corp., Brea, CA), marked Plate 1, 2, 3,... through 8 such that holder S3.1 is positioned with Plate 1, holder S3.2 is positioned with Plate 2, etc. The orientation is such that the columns (and therefore the rows)  
25 of the holders are aligned with the columns (and therefore the rows) of the plates. The dimensions of an array coverage in a plate would be from Rows A through H and from Columns 1 through 8 using the nomenclature common to the microtiter plate format. The crowns are treated with 5% trifluoroacetic acid in  
30 dichloromethane for 30 min. The crown holders are removed and the volatile contents of the microtiter plates evaporated under reduced pressure (20 mmHg).

The identity of any tripeptide is readily identified by its location in the microtiter plates. A code for deciphering the identity is  
35 readily constructed. If the M is the set of  $m$  amino acids used in the first step, N is the set of  $n$  amino acids used in the second step, and P is the set of  $p$  amino acids used in the third step; and,  $m = n = p = 8$ ; then the sets have the contents as follows:

$M \{ 1, 2, \dots, m \} = M \{ 1, 2, 3, \dots, 8 \} = M \{ \text{glycine, alanine, phenylalanine, leucine, lysine, glutamic acid, serine, threonine} \}$

$N \{ 1, 2, \dots, n \} = N \{ 1, 2, 3, \dots, 8 \} = N \{ \text{glycine, alanine, phenylalanine, leucine, lysine, glutamic acid, serine, threonine} \}$

5  $P \{ 1, 2, \dots, p \} = P \{ 1, 2, 3, \dots, 8 \} = P \{ \text{glycine, alanine, phenylalanine, leucine, lysine, glutamic acid, serine, threonine} \}$

10 The  $p$  arrays of dimension  $(m \times n)$  are physically located in Plates 1, 2, ..., 8, and the  $m$  and  $n$  dimensions in these Plates are defined by rows A-H and columns 1-8, respectively. Therefore, using the oligomer position and the de-coding nomenclature,  $M_m - N_n - P_p$ , the following locations have the following sequences (sequences read N-to-C as per peptide nomenclature):

Plate 1 Well A1, H-Gly-Gly-Gly-NH<sub>2</sub>;

Plate 4 Well A1, H-Leu-Gly-Gly-NH<sub>2</sub>;

15 Plate 2 Well C7, H-Ala-Ser-Phe-NH<sub>2</sub>;

Plate 6 Well E6, H-Glu-Glu-Lys-NH<sub>2</sub>;

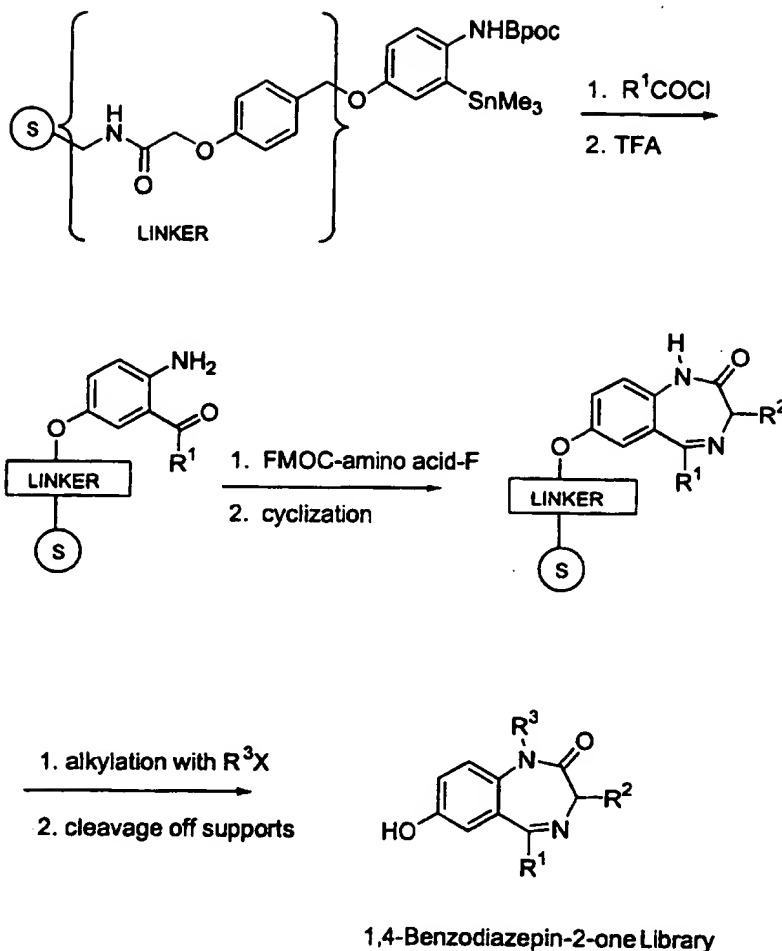
and so on.

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## II. A Library of 1536 1,4-Benzodiazepin-2-ones

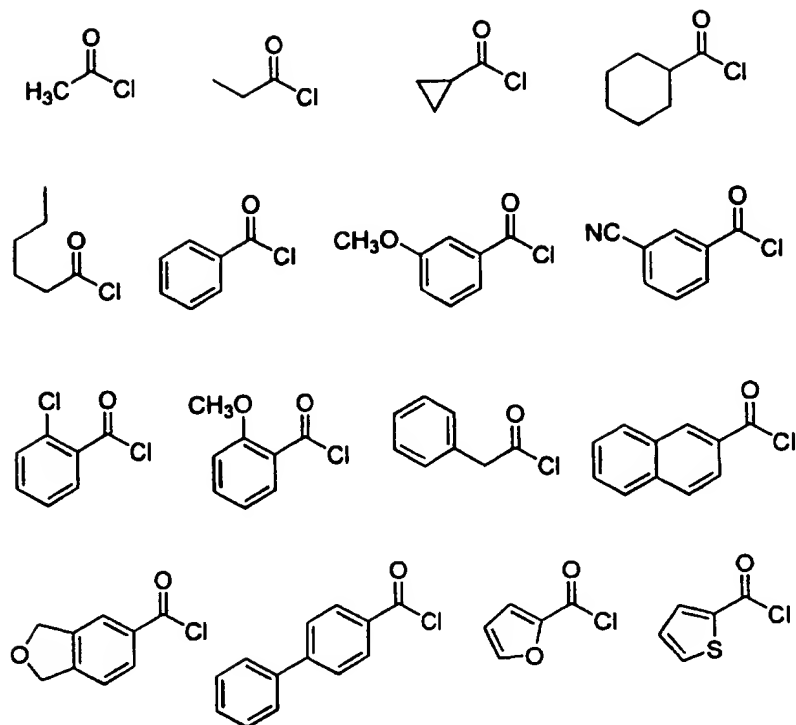
25 The methods described in the body of the invention may be used to produce non-peptide, low molecular weight organic compound libraries. The synthetic chemistry protocols are more complex in the syntheses of many of these compounds than those utilized to construct peptide libraries. Additionally, there are no general methods available to directly sequence the structure of most of these compounds. Thus, the ease by which a library is de-coded using the method described herein renders it suitable for the synthesis of low molecular weight compounds. The synthesis of a heterocyclic library is demonstrated. The synthesis of 1,4-benzodiazepin-2-ones follows the procedure of Ellman et. al.<sup>17</sup> and is shown in the accompanying schematic.

35

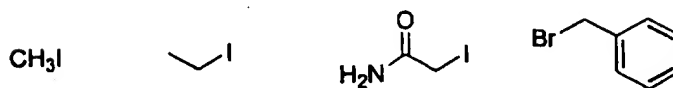


5

- 10 The procedure combines 16 acid chlorides (defining the variability at the position  $R^1$ ) and 24 amino acids (defining the variability at the position  $R^2$ ) with 4 alkylating agents (defining the variability at the position  $R^3$ ). These monomer groups are shown in the table below.

Acid ChloridesAmino Acid Fluorides

alanine(d and l)  
 aspartic acid(tert-Butyl ester)(d and l)  
 valine(d and l)  
 leucine(d and l)  
 glutamic acid(tert-Butyl ester)(d and l)  
 serine(tert-Butyl ether)(d and l)  
 phenylalanine(d and l)  
 tryptophan(d and l)  
 tyrosine(tert-Butyl ester)(d and l)  
 glutamine(d and l)  
 aspartamine(d and l)  
 ornithine(t-BOC)(d and l)

Alkylating Agents



A collection of 1536 solid supports in the form of aminomethylated polystyrene-grafted polyethylene crowns (Chiron Mimotopes Pty. LTD., Victoria, Australia) are required for the synthesis. A platform of crown holders to hold the necessary array sizes is constructed by ultrasonic welding four 96-position array crown holders on edge such that a 384-position platform of array dimension (16 x 24). Sixteen crown platforms uniquely marked with row and column positions clearly designated will be used for the first step in the synthesis, 24 platforms for the second step, and 4 platforms for the third step. The general method for redistributing arrays is followed as described for Case 1, in the Detailed Description of the Invention. Briefly, one constructs 16 arrays of crowns of dimension (24 x 4) on 16 crown platforms for the first step, rearranges the columns from the crown arrays in the first step to the columns of 24 arrays of dimension (16 x 4) on 24 crown platforms for the second step, then rearranges the rows of arrays from the second step to rows of 4 arrays of dimension (16 x 24) on 4 crown platforms for the third step.

The crowns are prepared analogous to the method of Ellman (JACS, 1995, 117, 3306-3307). The cyanomethyl ester of 4-(((4-Bpoc-amino-3-trimethylstannyl)-phenoxy)-methyl)-phenoxyacetic acid (250 mM) is reacted with the crowns in tetrahydrofuran for 4 hours. The crowns are filtered, washed with tetrahydrofuran, then with dichloromethane, dried, and mounted on the 16 crown platforms. The 16 crown platforms are placed in 16 reaction vessels which are trays as described heretofore. The 16 acid chlorides are coupled in the first step using the Stille coupling with the catalyst  $\text{Pd}_2(\text{dba})_3$ -chloroform. Following the coupling the arrays of crowns are washed with chloroform, exposed to a solution of 3% trifluoroacetic acid in dichloromethane for 5 min, then washed with dichloromethane and dried. The rearrangement of the arrays is then carried out as described. The 24 arrays of crowns on 24 platforms are then coupled with the 24 fluorenylmethyloxycarbonyl-amino acid fluorides (100mM) using 4-methyl-2, 6-tert-butylpyridine (400mM) in dichloromethane in 24 reaction vessels for 12 hours. The crowns are washed liberally with dichloromethane and then treated with a 20% solution of

5 piperidine in dimethylformamide for 40 min, washed with  
dimethylformamide, then dichloromethane, and dried. The crowns  
are then warmed in a solution of 5% acetic acid in  
dimethylformamide at 60°C overnight to cyclize the benzodiazepine  
ring. After washing liberally with dimethylformamide and  
dichloromethane and drying, the 24 arrays of crowns are  
redistributed from the second set of crown platforms to the 4  
crown platforms as described for the third step. The crown  
platforms are placed in 4 reaction vessels which are enveloped in  
dry argon in a glove box. The vessels are half-filled with dry THF,  
10 cooled to -78°C, and filled with a 200mM solution of lithiated 5-  
(phenylmethyl)-2-oxazolidinone in THF. After 1 hour a solution of  
the appropriate alkyl halide (100mM) in THF is added to each  
reaction vessel. The vessels are allowed to warm to room  
15 temperature, the reaction continuing for another 5 hours. The  
crowns are washed with THF and dried.

A 16 x 24 array of benzodiazepines will be released from each  
of the 4 platforms of crowns. Thus, an appropriately marked  
arrangement of 4 sets of four 96-well deep-well microtiter plates  
20 with 24 wells running across and 16 wells running down are  
positioned such that the individual crowns from each platform fit  
neatly into each well of the plates. The crowns are treated with  
85:5:10 trifluoroacetic acid/water/dimethylsulfide for 2 hours.  
The crown holders are removed and the volatile contents of the  
25 microtiter plates evaporated under reduced pressure (20 mmHg).

The identity of any individual 1,4-benzodiazepin-2-one is  
determined by its physical location in the four tetrameric  
arrangement of microtiter plates.

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We claim:

- 5           1. A method for producing a combinatorial library of oligomers which comprises:
- 10           a) calculating the sequence of positional transformations of solid supports among arrays which are required to produce the oligomers;
- 15           b) distributing a number of solid supports equal to the total number of oligomers to be synthesized into a first series of arrays;
- c) reacting a subset of monomers with the solid supports in a series of reaction vessels using a suitable chemical transformation, one array of supports per reaction vessel;
- 20           d) redistributing the first series arrays of solid supports into a second series of arrays such that all possible combinations of oligomers will be synthesized;
- e) reacting a new subset of monomers with the solid supports, in a series of reaction vessels using a suitable chemical transformation, one array of supports per reaction vessel;
- 25           f) identifying every individual oligomer in the library by analyzing the position of every solid support in each array at each step in the library synthesis.
2. A method according to claim 1. which further comprises:
- 30           a) redistributing the second series arrays of solid supports into a third series of arrays such that all possible combinations of oligomers will be synthesized;
- b) reacting a new subset of monomers with the solid supports, in a series of reaction vessels using a suitable chemical transformation, one array of supports per reaction vessel;
- 35           c) identifying every individual oligomer in the library by analyzing the position of every solid support in each array at each step in the library synthesis.

3. A method for producing a library of claim 2. of the quantity  $n \times m \times p$  oligomers, wherein  $m$ ,  $n$ , and  $p$  are integers, which comprises:

- a) calculating the sequence of positional transformations of solid supports among arrays which are required to produce the oligomers;
- b) distributing the quantity  $m \times n \times p$  solid supports into a first series of arrays of the dimension  $n \times p$  whereby each uniquely marked array contains  $n$  rows and  $p$  columns of solid supports and the total number of uniquely marked arrays is equal to  $m$ ;
- c) reacting a subset of  $m$  monomers with the solid supports in the series of  $m$  reaction vessels using a suitable chemical transformation, one array of supports per reaction vessel;
- d) redistributing the first series of arrays of solid supports into a second, but distinct series of arrays of the dimension  $n \times m$  such that each of the  $p$  columns from each of the  $m$  arrays of the first series are evenly distributed to form a column in each of  $p$  uniquely marked arrays of the second series, whereby each uniquely marked array of the second series contains  $n$  rows and  $m$  columns of solid supports;
- e) reacting a subset of  $p$  monomers with the solid supports in the series of  $p$  reaction vessels using a suitable chemical transformation, one array of supports per reaction vessel;
- f) redistributing the second series of arrays of solid supports into a third, but distinct series of arrays of the dimension  $p \times m$  such that each of the  $n$  rows from each of the  $p$  arrays of the second series are evenly distributed to form a row in each of  $n$  uniquely marked arrays of the third series, whereby each uniquely marked array of the third series contains  $p$  rows and  $m$  columns of solid supports;
- g) reacting a subset of  $n$  monomers with the solid supports in the series of  $n$  reaction vessels using a suitable

chemical transformation, one array of supports per reaction vessel;

- h) identifying every individual oligomer in the library by analyzing the position of every solid support in each array at each step in the library synthesis.

4. A method for producing a library of claim 2. of the quantity  $n \times m \times p$  oligomers, wherein  $m$ ,  $n$ , and  $p$  are integers, which comprises:

a) calculating the sequence of positional transformations of solid supports among arrays which are required to produce the oligomers;

b) distributing the quantity  $m \times n \times p$  solid supports into a first series of arrays of the dimension  $n \times p$  whereby each uniquely marked array contains  $n$  rows and  $p$  columns of solid supports and the total number of uniquely marked arrays is equal to  $m$ ;

c) reacting a subset of  $m$  monomers with the solid supports in the series of  $m$  reaction vessels using a suitable chemical transformation, one array of supports per reaction vessel;

d) redistributing the first series of arrays of solid supports into a second, but distinct series of arrays of the dimension  $m \times p$  such that each of the  $n$  rows from each of the  $m$  arrays of the first series are evenly distributed to form a row in each of  $n$  uniquely marked arrays of the second series, whereby each uniquely marked array of the second series contains  $m$  rows and  $p$  columns of solid supports;

e) reacting a subset of  $n$  monomers with the solid supports in the series of  $n$  reaction vessels using a suitable chemical transformation, one array of supports per reaction vessel;

f) redistributing the second series of arrays of solid supports into a third series of arrays of the dimension  $m \times n$  such that each of the  $p$  columns from each of the  $n$  arrays of the second series are evenly distributed to

form a column in each of  $p$  uniquely marked arrays of the third series, whereby each uniquely marked array of the third series contains  $m$  rows and  $n$  columns of solid supports;

5 g) reacting a subset of  $p$  monomers with the solid supports in the series of  $p$  reaction vessels using a suitable chemical transformation, one array of supports per reaction vessel;

10 h) identifying every individual oligomer in the library by analyzing the position of every solid support in each array at each step in the library synthesis.

15 5. A method according to claim 3. or claim 4. such that the redistribution of all the solid supports in any of each distinct row or each distinct column is accomplished in parallel.

20 6. A method according to claim 3. or claim 4. such that any two, or alternatively, all, of integers from the set  $\{ m, n, p \}$  may be equal in value.

25 7. A method according to claim 3. or claim 4. such that individual chemical transformations can be carried out on the solid supports, the monomers on the solid supports, the oligomers on the solid supports before, in between, and after steps a) through i).

30 8. A method according to claim 3. or claim 4. such that any subset of monomers may react with any single or multiple number of chemical structures present on the solid supports such that any related combination of linear, branched or cyclic products are formed in the library.

35 9. A method according to claim 3. or claim 4. such that the library of oligomers is linked to the solid supports via a cleavable linker.

10. A method according to claim 3. or claim 4. such that the library contains at least 100 oligomer members.

11. A method according to claim 10, such that the library contains between 1,000 to at least 100,000 oligomer members.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/04500

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/53

US CL :435/6, 7.1; 436/518

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1; 436/518

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN

search terms: peptide library, combinatorially library, array, addressable

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94/05394 A (ARRIS PHARMACEUTICAL CORPORATION) 17 March 1994, see page 6, line 18 - page 7, line 8, and page 11, line 4 - page 12, line 38.	1-11
A	BIRNBAUM et al. Peptide Screening. Current Opinion in Biotechnology. February 1992, Vol. 3, No. 1, pages 49-54, see entire document.	1-11
A	GALLOP et al. Applications of Combinatorial Technologies to Drug Discovery. 1. Background and Peptide Combinatorial Libraries. J. Med. Chem. 29 April 1994, Vol. 37, No. 9, pages 1233-1251, see entire document.	1-11

☐ Further documents are listed in the continuation of Box C., ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

15 MAY 1997

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